PATENT
Docket No.: **19603/3340 (CRF D-2018B)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	: Qiu et al.	**ATTENTION: DIRECTOR TC 1600**
Serial No.	: 09/597,840	
Cnfrm. No.	: 6516	Examiner: A. Kubelik
Filed	: June 20, 2000	
For	: ENHANCEMENT OF GROWTH IN PLANTS	Art Unit: 1638

PETITION UNDER 37 C.F.R. § 1.144
FOR REVIEW OF RESTRICTION REQUIREMENT

U.S. Patent and Trademark Office
Crystal Mall 1
1911 South Clark Place, 7th Floor
Arlington, Virginia 22202
ATTENTION: DIRECTOR TC 1600

Dear Center Director:

Pursuant to 37 C.F.R. § 1.144, applicants hereby petition for withdrawal of the restriction requirement imposed in the September 7, 2001, office action and made final in the March 27, 2002, office action. In their November 21, 2001, response to the restriction requirement, applicants elected the claims of Group II (i.e., claims 38, 39, 41, and 46-50), with traverse. For the following reasons, applicants respectfully submit that the restriction requirement is improper and should be withdrawn.

The claims of the present application (i.e., claims 38-51) are directed to methods of enhancing growth in plants by transformation with nucleic acids that encode hypersensitive response elicitors of plant pathogens. The claims also recite various plant pathogens from which the hypersensitive response elicitors are derived, including *Erwinia* spp., *Pseudomonas* spp., *Xanthomonas* spp., and *Phytophthora* spp. Further, the claims point out specific species of these plant pathogens that are encompassed by the present invention, including *Erwinia chysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, and *Xanthomonas campestris*.

The pending claims of the present application are related to one another in that they all involve transforming plants with a nucleic acid molecule encoding a hypersensitive response elicitor to enhance growth of the plant. Dependent claims 39-45 call for the nucleic acid molecule to be from various plant pathogens. Because these hypersensitive response elicitors are widely recognized in the art as a class or genus of related compounds, a restriction requirement based on the source of the hypersensitive response elicitor is inappropriate.

In plants, the hypersensitive response phenomena results from an incompatible interaction between plant pathogens and their non-host plants. As explained in Gopalan et al., "Bacterial Genes Involved in the Elicitation of Hypersensitive Response and Pathogenesis," Plant Disease 80: 604-10 (1996) ("Gopalan") (attached hereto as Exhibit 1), this interaction involves a bacterium attempting to infect a host plant, preventing multiplication and spreading of the pathogen, and collapse of plant leaf cells and cell death at the site of infection. This is distinct from a compatible interaction between bacteria and a plant where the bacteria spread in the infected plant, leading to disease symptoms throughout the plant. Id. at 604.

Genes controlling hypersensitive response elicitation and pathogenesis (i.e., *hrp* genes) are recognized in the art to be present in a limited class of Gram-negative pathogens, including *Erwinia, Pseudomonas, Burkholderia,* and *Xanthamonas* pathogens. See Bonas, "Bacterial Home Goal by Harpins," Trends Microbiol. 2: 1-2 (1994) ("Bonas I") (attached hereto as Exhibit 2); Bogdanove et al., "Unified Nomenclature for Broadly Conserved *hrp* Genes of Phytopathogenic Bacteria," Molec. Microbiol. 20:681-83 (1996) ("Bogdanove") (attached hereto as Exhibit 3); and Gopalan. These genes are found in large clusters of 20-40 kb and are conserved physically and functionally amongst different species. Bonas I, Bogdanove, and Gopalan.

Hypersensitive response elicitors have a number of common characteristics. These include their being glycine rich, heat stable, hydrophilic, capable of inducing a hypersensitive response in tobacco after recombinant expression, susceptible to proteolysis, and cysteine lacking. See Bonas, "*hrp* Genes of Phytopathogneic Bacteria," Current Topics in Microbiology and Immunology 192: 79-98 (1994) ("Bonas II") (attached hereto as Exhibit 4); Bonas I; and Preston et al., "The HrpZ Proteins of *Pseudomonas syringae* pvs. *syringae, glycinea,* and *tomato* are Encoded by an Operon Containing *Yersinia ysc* Homologs and Elicit the Hypersensitive Response in Tomato but not Soybean," MPMI 8(5): 717-32 (1995) ("Preston") (attached hereto as Exhibit 5).

The hypersensitive response elicitors within a given genus are also homologous to other elicitors from different pathogenic species of that genus. For example, *Erwinia amylovora* harpin is homologous to other *Erwinia* spp. harpins. See Willis et al., "*hrp* Genes of Phytopathogenic Bacteria," MPMI 4(2): 132-38 (1991) ("Willis") (attached hereto as Exhibit 6); Ahmad et al., "Harpin Is Not Necessary for the Pathogenicity of Maize," 8th Int'l Cong. Molec. Plant Microbe Inter. July 14-19, 1996 ("Ahmad I") (attached hereto as Exhibit 7); and Gopalan. In addition, *Pseudomonas syringae* pv. *syringae* harpin is homologous to other *Pseudomonas syringae* harpins. See Gopalan; Preston; and Willis. Different *Pseudomonas solancearum* harpins have also been found to be homologous to one another. Arlat et al., "PopA1, a Protein which Induces a Hypersensitivity-like Response on Specific *Petunia* Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solancearum,*" EMBO J. 13(3): 543-53 (1994) ("Arlat") (attached hereto as Exhibit 8) and Willis.

Further, genes encoding hypersensitive response elicitors from different genera have been found to be similar. For example, the *Xanthamonas campestris* and *Pseudomonas solancearum* harpin encoding genes are similar to one another, while those from *Erwinia amylovora* and *Pseudomonas syringae* are also similar. Van Gijsegem et al., "Conservation of Secretion Pathways for Pathogenicity Determinants for Plant and Animal Bacteria," Trends Microbiol. 1: 175-80 (1993) ("Van Gijsegem") (attached hereto as Exhibit 9) and Bogdanove.

In Bauer et al., "*Erwinia chrysanthemi* Harpin$_{Ech}$: An Elicitor of the Hypersensitive Response that Contributes to Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995) ("Bauer et al. 1995") (attached hereto as Exhibit 10), the *Erwinia amylovora* hypersensitive response elicitor encoding gene was used as a probe to isolate, clone, and sequence the gene coding for the *Erwinia chrysanthemi* hypersensitive response elicitor as follows:

> The cosmids were probed in colony blots with a 1.3-kb *Hind*III fragment from pCPP1084, which contains the *E. amylovora hrpN* gene (Wei et al. [, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (]1992[)]). pCPP2157, one of the three cosmids hybridizing with the probe, was digested with several restriction enzymes, and the location of the *hrpN$_{Ech}$* gene in those fragments was determined by probing a Southern blot with *E. amylovora Hind*III fragment. Two fragments, each containing the entire *hrpN$_{Ech}$* gene, were subcloned into different vectors: pCPP2142 contained an 8.3-kb *Sal*I fragment in pUC119 (Vieira and Messing [,"Production of Single-Stranded Plasmid DNA," Methods Enzymol., 153:3-11(] 1987[)]), and pCPP2141 contained a 3.1-kb *Pst*I fragment in pBluescript II SK(-) (Stratagene, La Jolla, CA).

> ***Sequence of $hrpN_{Ech}$***
>
> The nucleotide sequence of a 2.4-kb region of pCPP2141 encompassing $hrpN_{Ech}$ was determined. The portion of that sequence extending from the putative ribosome-binding site through the $hrpN_{Ech}$ coding sequence to a putative rho-independent terminator is presented in Figure 1.

See page 485. As noted in Bauer et al., "*Erwinia chrysanthemi hrp* Genes and Their Involvement in Soft Rot Pathogenesis and Elicitation of the Hypersensitive Response," MPMI 7(5): 573-81 (1994) ("Bauer et al. 1994") (attached hereto as Exhibit 11), a probe carrying a fragment of the *Erwinia amylovora* hypersensitive response elicitor encoding gene not only hybridizes to the gene encoding the hypersensitive response elicitor for *Erwinia chrysanthemi* but also to the gene encoding the hypersensitive response elicitor for *Pseudomonas syringae* p.v. *syringae*. See abstract and pages 574 and 576. In addition, Cui et al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress $hrpN_{Ecc}$ and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996) ("Cui") (attached hereto as Exhibit 12) further indicates that the gene encoding the *Erwinia carotovora* hypersensitive response elicitor can be isolated, sequenced, and cloned by using the *Erwinia chrysanthemi* hypersensitive response elicitor encoding gene to probe the genomic library of *Erwinia carotovora* in the same manner as that probing gene was found from the *Erwinia amylovora* hypersensitive response elicitor encoding gene, as discussed *supra*. Further, Cui (at page 572) reads as follows:

> The genomic library of *E. carotovora* subsp. *carotovora* strain Ecc71 in pLARF5 was screened by in situ colony hybridization with a 0.75-kb internal *Cla*I fragment of *hrpN* of *E. chrysanthemi* (Bauer et al. 1995). Two cosmids, pAKC921 and pAKC922, that hybridized with the probe were isolated. The subclones (pAKC923 and pAKC924, Table 1) carrying *hrpN* DNA were used for sequence analysis.

The gene encoding the hypersensitive response elicitor of *Erwinia amylovora* has also been used as a probe to isolate and clone the gene encoding the hypersensitive response elicitor of *Erwinia stewartii*. It was additionally found that antibodies raised against the hypersensitive response elicitor of *Erwinia stewartii* cross-reacted with the hypersensitive response elicitor of *Erwinia amylovora*. See Ahmad I and Ahmad et al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996 ("Ahmad II") (attached hereto as Exhibit 13).

Further, the hybridization studies in Laby et al., "Hybridization and Functional Complementation of the *hrp* Gene Cluster from *Erwinia amylovora* Strain Ea321 with DNA

of Other Bacteria," Mol. Plant-Microb. Inter. 5: 412-19 (1992) ("Laby") (attached hereto as Exhibit 14) indicate that probes from the hypersensitive response elicitor encoding gene cluster for *Erwinia amylovora* hybridized to genomic DNA from other *Erwinia* species and from *Pseudomonas syringae* species.

Finally, submitted simultaneously with this petition is an Amendment and a Declaration of Zhong-Min Wei under 37 C.F.R. § 1.132 (Wei Declaration"). In paragraphs 10-11 of the Wei Declaration, the topical application of the hypersensitive response elicitors from *Xanthomonas campestris* and *Pseudomonas syringae* is shown to enhance growth. Thus, these elicitors achieve the same results as those set forth in the examples of the present application where the hypersensitive response elicitor is from *Erwinia amylovora*. This further demonstrates that hypersensitive response elicitors are an art recognized class of compounds.

From all of the foregoing literature, there is ample support to show that hypersensitive response elicitors from plant pathogens are a well known phenomenon caused by a limited number of plant pathogens. In view of the similarity amongst the hypersensitive response eliciting proteins of different pathogenic species, as well as the similarity of the genes encoding the various hypersensitive response elicitors, these elicitors clearly constitute an art recognized class of compounds. On this basis alone, it is clear that the restriction requirement issued in this case is improper.

Further, the U.S. Patent and Trademark Office ("USPTO") has failed to satisfy its burden of demonstrating that the claimed inventions are unrelated. In the September 7, 2001, office action, the USPTO recited as the appropriate test: "[i]nventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects" (citing MPEP §§ 806.04, 808.01) (emphasis added). However, the USPTO has failed to demonstrate that the inventions actually have different modes of operation, different functions, or different effects. Clearly, the function of each claimed method is to enhance plant growth; thus, each claimed method has the same function and effect. The USPTO has asserted that since different hypersensitive response elicitors have different nucleic acid coding sequences, they must involve different modes of operation. However, the USPTO provided no evidence to support this assertion. In light of the above demonstration that hypersensitive response elicitors are an art-recognized class of compounds, the opposite seems to be true – i.e., their mode of action is similar. In any event, since the USPTO has clearly failed to carry its burden, the restriction requirement is improper and should be withdrawn.

Given the presence of generic linking claims and a sub-genus Markush group which recites the use of nucleic acid molecules encoding hypersensitive response elicitor proteins or polypeptides derived from different plant pathogens, the proper course in the instant application is to include the claimed inventions of Groups I-VIII together in a single group, requiring only an election of species at this time. See MPEP § 808.01(a). This procedure is proper for applications containing generic or Markush-type claims, which is exactly the situation here.

Finally, applicants question the proposed classification of each group asserted in the outstanding restriction requirement. It appears the USPTO recited different class/subclass designations for each group of invention as support for the proposition that examination of more than one group would constitute a burden to the USPTO. In reviewing the on-line USPTO classification manual, however, it appears (in most instances) each group of invention would be searchable under almost all of the cited class/subclass designations. There appears to be no basis for including one group in a particular class/subclass while excluding others.

As one example, the USPTO indicated that the invention of Group I, limited to the claimed method where the hypersensitive response elicitor is derived from *Erwinia chrysanthemi*, would be classified in class 800, subclass 278. The definition for class 800, subclass 278, recites: "METHOD OF INTRODUCING A POLYNUCLEOTIDE MOLECULE INTO OR REARRANGEMENT OF GENETIC MATERIAL WITHIN A PLANT OR PLANT PART: This subclass is indented under the class definition. Method for insertion of polynucleotide molecules into, or rearrangement of genetic material within a plant cell, wherein said cell is part of, or regenerated into, a plant or plant part." Based on this definition, there does not appear to be any basis for distinguishing Group I from any of Groups II-VII. Therefore, this class/subclass should be searched for each group of invention.

As another example, the USPTO indicated that the invention of Group II, limited to the claimed method where the hypersensitive response elicitor is derived from *Erwinia amylovora*, would be classified in class 536, subclass 23.7. The definition for class 536, subclass 23.7 recites: "Encodes a microbial polypeptide: This subclass is indented under subclass 23.1 [DNA or RNA fragments or modified forms thereof]. Compounds which are DNA fragments which encode specific microbial polypeptides." Based on this definition, there does not appear to be any basis for distinguishing Group II from Groups I and III-VIII in this regard. Therefore, this class/subclass should be searched for most groups of invention.

In yet another example, the USPTO indicated that the invention of Group III, limited to the claimed method where the hypersensitive response elicitor is derived from

Pseudomonas syringae, would be classified in class 800, subclass 298. The definition for class 800, subclass 298 recites: "Higher plant, seedling, plant seed, or plant part (i.e., angiosperms or gymnosperms): This subclass is indented under subclass 295 [plant, seedling, plant seed, or plant part per se]. Subject matter wherein the plant, seedling, plant seed, or plant part is a higher plant, i.e., an angiosperm or gymnosperm, both of which produce seeds." Based on this definition, there does not appear to be any basis for distinguishing Group III from Groups I-II and IV-VII. Therefore, this class/subclass should be searched for each group of invention.

As a further example, the USPTO indicated that the invention of Group IV, limited to the claimed method where the hypersensitive response elicitor is derived from *Pseudomonas solanacearum*, would be classified in class 800, subclass 288. The definition for class 800, subclass 288 recites: "Nonplant protein is expressed from the polynucleotide: This subclass is indented under subclass 278 [see above definition]. Method wherein the polynucleotide encodes a polypeptide not originating from a plant." Based on this definition, there does not appear to be any basis for distinguishing Group IV from Groups I-III and V-VII. Therefore, this class/subclass should be searched for each group of invention.

In yet another example, the USPTO indicated that the invention of Group V, limited to the claimed method where the hypersensitive response elicitor is derived from *Xanthomonas campestris*, would be classified in class 435, subclass 468. The definition for class 435, subclass 468 recites: "Introduction of a polynucleotide molecule into or rearrangement of a nucleic acid within a plant cell: This subclass is indented under subclass 440 [PROCESS OF MUTATION, CELL FUSION, OR GENETIC MODIFICATION]. Processes of inserting polynucleotide molecules into or rearranging genetic material within a plant cell." Based on this definition, there does not appear to be any basis for distinguishing Group V from Groups I-IV and VI-VII. Therefore, this class/subclass should be searched for each group of invention.

In a still further example, the USPTO indicated that the invention of Group VI, limited to the claimed method where the hypersensitive response elicitor is derived from *Phytophthora*, would be classified in class 435, subclass 419. The definition for class 435, subclass 419 recites: "Plant cell or cell line, per se, contains exogenous or foreign nucleic acid: This subclass is indented under subclass 410 [Plant cell or cell line, per se]. Subject matter wherein the plant cell or cell line has been transformed by the insertion of nucleic acid which is either exogenous or foreign to it." Based on this definition, there does not appear to be any basis for distinguishing Group VI from Groups I-V and VII. Therefore, this class/subclass should be searched for each group of invention.

In a final example, the USPTO indicated that the invention of Group VII, limited to the claimed method where growth enhancement is achieved by topical application of a hypersensitive response elicitor to transgenic plants transduced with a hypersensitive response elicitor encoding gene. Applicants submit that this group is generic to use with a hypersensitive response elicitor encoding gene from any source. Further, the subject matter of Group VII would be classified in class 800, subclass 290 having the following definition: "The polynucleotide alters plant part growth (e.g., stem or tuber length, etc.) This subclass is indented under subclass 278 [see above definition]. Method wherein the polynucleotide causes the plant or plant part to be larger or smaller or to grow at a faster or slower rate than in the absence of said polynucleotide." Based on this definition, there does not appear to be any basis for distinguishing Group VII from Groups I-VI. Therefore, this class/subclass should be searched for each group of invention.

In view of the foregoing, it is apparent that the restriction requirement is improper and, therefore, should be withdrawn in its entirety.

Applicants believe that no fee is required for filing this petition. If additional fees are required, however, the Commissioner is hereby authorized to charge any fees to Deposit Account No. 14-1138.

Respectfully submitted,

Date: September 27, 2002

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Date 9/27/2002 JoAnn Whalen